CPMA®
COLOR PIGMENTS MANUFACTURERS ASSOCIATION, INC.

201-16819

September 15, 2009

Mark W. Townsend, Chief
HPV Chemicals Branch
Environmental Protection Agency
Office of Pollution Prevention
and Toxics
1201 Constitution Avenue, NW
Washington, DC 20004

Re: Response to EPA Comments on the CPMA Test Plan for C. I. Pigment Violet 19 (Chemical Abstracts Service ("CAS") Number 1047-16-1, C.I. Pigment Red 122, CAS No. 980-26-7 and Dyhydroquinacridone, CAS No. 5862-38-4

Dear Mr. Townsend:

I am writing on behalf of the Color Pigments Manufacturers Association, Inc. ("CPMA") in response to your letter of April 21, 2009 in which you provided the Environmental Protection Agency's ("EPA") comments on the CPMA Test Plan (the "Test Plan") and robust summaries for C.I. Pigment Red 122, Quino(2,3-b)acridine-7,14-dione,5,12-dihydro-2,9-dimethyl Chemical Abstracts Service ("CAS")

300 North Washington Street, Suite 105 • Alexandria, Virginia 22314 • USA
Phone: (703) 684-4044 • Fax: (703) 684-1795 • e-mail: cpma@cpma.com

No. 980-26-7); C.I. Pigment Violet 19, Quino(2,3-b)acridine-7,14-dione,5,12-dihydro, CAS No. 1047-16-1,(jointly the "Quinacridone Pigments") and Dihydroquinacridone, CAS NO. 5862-38-4. The Test Plan was submitted to the EPA as part of the voluntary High Production Volume ("HPV") testing program.

The CPMA is an industry trade association representing color pigment companies in Canada, Mexico, and the United States. CPMA represents small, medium, and large color pigments manufacturers throughout Canada, Mexico and the United States, accounting for the bulk of the production of color pigments in North America. Color pigments are widely used in product compositions of all kinds, including paints, inks, plastics, glass, synthetic fibers, ceramics, colored cement products, textiles, cosmetics, and artists' colors. Color pigment manufacturers located in other countries with sales in Canada, Mexico, and the United States and suppliers of intermediates, other chemicals and other products used by North American manufacturers of color pigments are also members of the Association.

Quinacridone Pigments

As discussed in our letter of July 20, 2009, CPMA has reviewed the Test Plan for the quinacridone pigments. In response to EPA's concerns, we are providing the enclosed robust summaries. These summaries provide more detailed descriptions of the study summaries previously submitted to EPA. A new summary for an environmental degradation study identified by our members is included for your review. We have also enclosed a new summary for an octanol water partition coefficient study completed using experimental results for octanol and water solubility. The enclosed summaries cover the following study endpoints:

- Acute Oral Toxicity in Rats
- Subchronic Oral Toxicity in Rats
- Mutagenicity Evaluation in the Ames Salmonella/Microsome Plate Test
- In Vivo-In Vitro Rat Hepatocyte Unscheduled DNA Synthesis Assay
- An In-Vivo Cell Mutation Assay
- A Radio labeled Tracer Study of C QV 19 in the Albino Rat Dosed by Oral Gavage
- Quantitative Digital Image Analysis of Whole body Autoradiographic Localization Following Administration of C-QV 19 in the Albino Rat Dosed by Oral Gavage
- Environmental Degradation (OECD 301C)
- Octanol and water solubility and measured Log\Kow
- 21 Day Daphnia Magna Reproduction

Dihydroquinacridone

With respect to the intermediate dihydroquinacridone, we have reviewed the current generation and use of this substance in commerce with our members. The only known use of dihydroquinacridone is as an intermediate in the production of quinacridone pigments. Dihydroquinacridone is completely reacted by oxidation to form finished quinacridone pigments, such as C.I. Pigment Violet 19. Quinacridone pigments do not contain residual dihydroquinacridone. To our knowledge, there is no market for or sale of isolated dihydroquinacridone in commerce and there is no potential for consumer or downstream commercial exposure to dihydroquinacridone.

It is our understanding that dihydroquinacridone is only generated or used in no more than two facilities in the United States. Although we are not aware of any significant hazard posed by dihydroquinacridone, we have discussed the use and exposure presented by this chemical with our member companies. There are fewer than ten employees total in the United States which have any potential exposure to dihydroquinacridone. These potential

exposures are carefully minimized using engineering controls and, if necessary, personal protective equipment.

Conclusion

CPMA makes no commitment with respect to the quinacridone Pigments and the intermediate dihydroquinacridone discussed above or any guideline or requirement established pursuant to the voluntary HPV program or otherwise. Furthermore, CPMA reserves the right to defer the review of this chemical if it or an analog has been the subject of another undertaking in any EPA program or other similar international programs.

CPMA further reserves the right to withdraw the Test Plan should the HPV program, when and if finalized, prove to be different from that understood, from time to time, by CPMA. Since all of the pigments and intermediates represented by CPMA have been used in international commerce for many years, there is extensive data available from a variety of published and unpublished sources.

Thank you for your attention to this matter. Please call me if there are any questions or comments.

Sincerely,

J. Lawrence Robinson
President

A1. Genetic Toxicity - Mutation

Test substance:	Quinacridone Red Y, C. I. Pigment Violet 19 CAS No. 1047-16-1
Remarks:	Lot# 566951

Method

Method:	Invitro microbial mutagenicity
Test type:	Ames (DMT-100)
GLP:	No
Year:	1979
Species/strain:	Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538,
Metabolic activation:	With and without S9-Mix
Concentration tested:	10 ug -100 ug/plate (Standard Plate Test)

Procedure:

Scope of Tests and Conditions:
TA 98, TA 100, TA 1535, TA 1537, TA 1538
Doses:.5 ; 1.0; 10.0; 100; 500; 1000 ug/plate
Vehicle: DMSO
Standard Plate Test with and without S-9 Mix
DMSO was selected as the vehicle.

Metabolic Activation:

TPN (Sodium Salt) 4µmol
Glucose-6-phosphate 5µmol
Sodium Phosphate (dibasic) 100µmol
MgCl2 8µmol
KCl 33µmol
Homogenate S9 fraction .1+/- .05 ml

S9 Homogenate

S-9 Fraction and S-9 Mix contained .3 ml of 9000 Xg supernatent was prepared from sprague dawley adult male rat liver induced by Aroclor 1254 five days prior to kill according to procedure of Ames et al. 1975. S9 samples were coded by lot number and assayed for milligrams protein per millitliter and relative P448/ P450 activity by methods described in LBI Technical Data on Rat liver S9 product.

Incubation at 37° C for 48 hours.

No deviation noted from the standard guideline.

The test chemical is considered positive in this assay if the following criteria are met:

1.0 Strains TA1535, TA 1537 and TA 1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2.0 Strains TA 98 and TA 100

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA 100 and 2-3 time the solvent control value strain TA 98 is considered to be mutagenic. For these strains, the dose-response increase should start at approximately the solvent control value.

Controls:

Negative controls, solvent DMSO and control plate without S9 activators.

Positive Controls:

With Activation:

2- anthramine 2.5 ug/plate all strains- TA 98, TA 100, TA 1535, TA 1537, TA 1538

Without Activation:

TA 98	2-Nitrofluorene 10 ug/plate
TA 100	Sodium Azide 1 ug/plate
TA 1535	Sodium Azide 1 ug/plate
TA 1537	9-Aminoacridine 50 ug/plate
TA 1538	2-Nitrofluorene 10 ug/plate

RESULTS

STANDARD PLATE TESTS

TA1535 Without S-9 Mix

Dose/Plate ug/plate	RevertantsPer Plate
DMSO	12
.50	13
1.0	12
10.0	18
100.0	15
500.0	10
1000.0	20
P.Contrl	145

TA1535 With S-9 Mix (1:9)

Dose/Plate ug/plate	Revertants Per Plate
DMSO	18
.5	12
1.0	6
10	10
100	9
500	10
1000	9
P.Cntrl.	159

TA1537 Without S-9 Mix

Dose/Plate ug/plate	Revertants Per Plate
DMSO	8
.50	5
1.0	9
10.0	7
100.0	12
500.0	2
1000.0	1
P.Contrl	1454

TA1537 With S-9 Mix (1:9)

Dose/Plate ug/plate	Revertants Per Plate	
	T/1	T/2
DMSO	27	8
.5	37	6
1.0	31	9
10	41	6
100	39	6
500	36	7
1000	17	3
P.Cntrl.	319	153

TA1538 Without S-9 Mix

Dose/Plate ug/plate	Revertants Per Plate
DMSO	13
.50	13
1.0	13
10.0	13
100.0	11
500.0	7
1000.0	8
P.Contrl	1186

TA1538 With S-9 Mix (1:9)

Dose/Plate ug/plate	Revertants Per Plate
DMSO	32
.5	29
1.0	28
10	23
100	27
500	29
1000	11
P.Cntrl.	304

TA98 Without S-9 Mix

Dose/Plate ug/plate	RevertantsPer Plate
DMSO	28
.50	22
1.0	22
10.0	21
100.0	26
500.0	26
1000.0	18
P.Contrl	1002

TA98 With S-9 Mix (1:9)

Dose/Plate ug/plate	Revertants Per Plate
DMSO	33
.5	39
1.0	35
10	41
100	40
500	37
1000	37
P.Cntrl.	1627

TA100 Without S-9 Mix		TA100 With S-9 Mix (1:9)	
Dose/Plate ug/plate	Mean Revertants	Dose/Plate ug/plate	Mean Revertants
DMSO	301	DMSO	320
.50	268	.5	182
1.0	251	1.0	296
10.0	121	10	339
100.0	231	100	344
500.0	304	500	340
1000.0	225	1000	242
P.Contrl	1129	P.Cntrl.	1778

Standard plate test, no increase in number of his or trp revertants for the strains TA 1535, TA100, TA 1537, TA 98, TA1538, with and without S-9 mix.

Cytotoxic concentration: No bacteriotoxic effect was noted at any dose.

Precipitation concentration: Precipitation found from about 200 ug/Plate

Remarks: The test substance did not significantly increase the spontaneous or background mutation frequency either without S-9 mix or after adding a metabolizing system.

Conclusions The test material is not a mutagenic agent in this bacterial reverse mutation test.

Data Quality

Reliability: Klimisch Code - 1- Reliable without restriction

Remarks:

References

Other

A1. Genetic Toxicity - Mutation

Test substance:	Monastral Violet R, C. I. Pigment Violet 19 CAS No. 1047-16-1
Remarks:	100% pure

Method

Method:	Invitro microbial mutagenicity
Test type:	Ames
GLP:	No
Year:	1975
Species/strain:	Salmonella typhimurium TA 1535, TA 1537, TA 1538,
Metabolic activation:	With and without S9-Mix
Concentration tested:	10 ug - 200 ug/plate (Standard Plate Test)

Procedure:	Scope of Tests and Conditions: TA 1535, TA 1537, TA1538, Doses:1 0;25;50;100;200;ug/plate Vehicle: DMSO Standard Plate Test with and without S-9 Mix DMSO was selected as the vehicle.

Metabolic Activation, S-9 Fraction and S-9 Mix contained .3 ml of 9000 Xg supernatent of homogenized rat liver, 8mm MgCl2 , 33mM NADP and 100MM sodium phosphate (pH 7.4) This mixture was added directly to the top agar immediately before it was poured over the minimal agar plate. The upper limit of the dose range was determined by the insolubility of the compound in the agar and not its toxicity to the tester strains. At the maximum dose the sample was a suspension of the chemical in the agar and not a true solution.

Incubation at 37° C for 48 hours.

No deviation noted from the standard guideline.

The test chemical is considered positive in this assay if the following criteria are met:

A statistically significant dose related increase in the number of revertant colonies is obtained in two separate experiments, and (2) the increase in the number of revertant colonies is at least twice the concurrent solvent control value.

Controls:

Negative controls, solvent DMSO and control plate without S9 activators.

Positive Controls used to check the mutability of the bacteria and the activity of the S-9 mix. With S-9 mix: 2 - aminoanthracene ("2AA") -2.0 ug/plate, dissolved in DMSO,

Without S-9 Mix;2- nitrofluorene("2NF") TA1538; 9-aminoacridine ("9AA"), Strain: TA1537, n-

methyl-N'-Nitro-N-Nitrosoguanidine.("MNNG") Strain TA 1535

RESULTS

STANDARD PLATE TESTS

TA1535 Without S-9 Mix

Dose/Plate ug/plate	Mean Revertants
DMSO	23
10	20
25	22
50	18
100	22
200	23
MNNG 1.0UG	~700
9AA	~1200

TA1535 With S-9 Mix (1:9)

Dose/Plate ug/plate	Mean Revertants
DMSO	8
S9 Cntrl	13
10	8
25	8
50	10
100	2
200	6
2AA 100	198

TA1537 Without S-9 Mix

Dose/Plate ug/plate	Mean Revertants
DMSO	8
10	9
25	7
50	13
100	9
200	8
9AA	~1200

TA1537 With S-9 Mix (1:9)

Dose/Plate ug/plate	Mean Revertants
DMSO	9
S9 Cntrl	11
10	6
25	5
50	5
100	17
200	12
2AA 100	778

TA1538 Without S-9 Mix

Dose/Plate ug/plate	Mean Revertants
DMSO	21
10	17
25	21
50	23
100	12
200	18
2nF	~4000

TA1538 With S-9 Mix (1:9)

Dose/Plate ug/plate	Mean Revertants
DMSO	19
S9 Cntrl	13
10	16
25	12
50	12
100	17
200	18
2AA 10	~4000

Standard plate test, no increase in number of his or trp revertants for the strains TA 1535, TA100, TA 1537, TA1538, 8 with and without S-9 mix.

Cytotoxic concentration: No bacteriotoxic effect was noted at any dose.

Precipitation concentration: Precipitation found from about 200 ug/Plate onward

Remarks: The test substance did not significantly increase the spontaneous or background mutation frequency either without S-9 mix or after adding a metabolizing system.

Conclusions The test material is not a mutagenic agent in this bacterial reverse mutation test.

Data Quality

Reliability: Klimisch Code - 1- Reliable without restriction

Remarks:

References

Other

A1. Genetic Toxicity - Mutation

Test substance:	Monastral Violet R, C. I. Pigment Red 19
Remarks:	CAS 1047-16-1, 100% pure

Method

Method:	Invitro microbial mutagenicity
Test type:	Ames
GLP:	No
Year:	1975
Species/strain:	Salmonella typhimurium TA 1535, TA 1537, TA 1538,
Metabolic activation:	With and without S9-Mix
Concentration tested:	10 ug -100 ug/plate (Standard Plate Test)

Procedure:	Scope of Tests and Conditions: TA 1535, TA 1537, TA1538, Doses:1 0;25;50;100; ug/plate Vehicle: DMSO Standard Plate Test with and without S-9 Mix DMSO was selected as the vehicle.

Metabolic Activation, S-9 Fraction and S-9 Mix contained .3 ml of 9000 Xg supernatent of homogenized rat liver, 8mm MgCl2 , 33mM NADP and 100MM sodium phosphate (pH 7.4) This mixture was added directly to the top agar immediately before it was poured over the minimal agar plate. The upper limit of the dose range was determined by the insolubility of the compound in the agar and not its toxicity to the tester strains. At the maximum dose the sample was a suspension of the chemical in the agar and not a true solution.

Incubation at 37° C for 48 hours.

No deviation noted from the standard guideline.

The test chemical is considered positive in this assay if the following criteria are met:

A statistically significant dose related increase in the number of revertant colonies is obtained in two separate experiments, and (2) the increase in the number of revertant colonies is at least twice the concurrent solvent control value.

Controls:

Negative controls, solvent DMSO and control plate without S9 activators.

Positive Controls used to check the mutability of the bacteria and the activity of the S-9 mix. With S-9 mix: 2 - aminoanthracene ("2AA") -2.0 ug/plate, dissolved in DMSO,

Without S-9 Mix;2- nitrofluorene("2NF") TA1538; 9-aminoacridine ("9AA"), Strain: TA1537, n-methyl-N'-Nitro-N-Nitrosoguanidine.("MNNG") Strain TA 1535

RESULTS

STANDARD PLATE TESTS

TA1535 Without S-9 Mix

Dose/Plate ug/plate	Mean Revertants
DMSO	17
10	15
25	11
50	17
75	18
100	19
MNNG 1.0UG	636

TA1535 With S-9 Mix (1:9)

Dose/Plate ug/plate	Mean Revertants
DMSO	26
S9 Cntrl	18
10	14
25	6
50	10
75	10
100	9
2AA 100	301

TA1537 Without S-9 Mix

Dose/Plate ug/plate	Mean Revertants
DMSO	13
10	9
25	7
50	7
75	15
100	9
9AA	~900

TA1537 With S-9 Mix (1:9)

Dose/Plate ug/plate	Mean Revertants
DMSO	10
S9 Cntrl	17
10	6
25	9
50	15
75	13
100	17
2AA 100	673

TA1538 Without S-9 Mix

Dose/Plate ug/plate	Mean Revertants
DMSO	14
10	20
25	15
50	12
75	21
100	18
2nF	~4000

TA1538 With S-9 Mix (1:9)

Dose/Plate ug/plate	Mean Revertants
DMSO	26
S9 Cntrl	17
10	16
25	17
50	19
75	21
100	10
2AA 10	~4000

Standard plate test, no increase in number of his or trp revertants for the strains TA 1535, TA100, TA

1537, TA1538, with and without S-9 mix.

Cytotoxic concentration: No bacteriotoxic effect was noted at any dose.

Precipitation concentration: Precipitation found from about 200 ug/Plate

Remarks: The test substance did not significantly increase the spontaneous or background mutation frequency either without S-9 mix or after adding a metabolizing system.

Conclusions The test material is not a mutagenic agent in this bacterial reverse mutation test.

Data Quality

Reliability: Klimisch Code - 1- Reliable without restriction

Remarks:

References

Other

C.Biodegradation

Test Substance	2,8 Dimethyl-5,12-dihydroquino [2,3b]acridine-7,14-dione
Test substance:	CAS No. 980-26-7
Remarks:	

Method

Method:	OECD 301C
Test type:	Standard
GLP:	
Year:	2005
Remarks:	4 week test period Chemical concentration 100 mg/L Concentration of activated sludge 10 mg/L A gravimetric method was used to directly quantify the test substance because there was no solvent to dissolve it.

Results

Results:	Substance was determined to be persistent
Remarks:	BOD analysis -3.0 -3.0,-4.0, (0.0) Direct analysis of Measured Weight 6.0, 4.0, 0 (3)%

Conclusions

Well documented study undertaken by a government agency.

Data Quality

Remarks: Klimishe Code 1 Reliable without restriction

References Japanese Official Bulletin of the Ministry of International Trade and Industry

Other

Genetic Toxicity

Test Substance

Test substance: 4, 11 Dichloroquinacrdone, CAS No. 3089-16-5

Remarks:

Method

Method: OECD 476
Test type: Cell Mutation Assay at the Thymidine Kinase Locus In Mouse Lymphoma L5178Y Cells
GLP: Yes
Year: 2000
Species/strain:
Exposure period: 4 and 24 hours
Remarks: Procedure :
- The assay was performed in two indeendent experiments, using two parallel cultures each. The first main experiment was performed with and without liver microcomal activation and a treatment period of 4 hours. The second experiment was soeley performed in the absence of metabolic activation with a treatment period of 24 hours.

Precipitation was cvisible to the unaided eye at 39.1 ug/ml and above with and without metabolic activation. No relevant toxic effects were observed up to the maximal analysable concentration of 312.5 ug/ml. Higher concentrations led to very heavy precipitation of the test item in the suspension cell cultures precluding cell counting.

DCQA was evaluated at the following concentrations:

Experiment 1

Without S9 mix: 5.0;10.0;20.0; 100.0; and 320.0 ug/ml
With S9 mix: 5.0;10.0;20.0; 100.0; and 320.0 ug/ml

Experiment 2

Without S9 mix: 5.0;10.0;20.0; 100.0; and 320.0 ug/ml

Solvent used was DMSO

Positive Controls:

With Metabolic Activation: 3-methycholanthrene
Without Metalbolic Activation: Methylmethane sulfonate

Negative Controls:

Concurrent negative and solvent controls were performed

Results:

conc.ug/ml	culture 1 relative cloning efficiency	relative total growth	mutant colonies 10^6 cells	induction factor	culture 2 relative cloning efficiency	relative total growth	mutant colonies 10^6 cells	induction factor
Experiment 1								
Ng.cntr.	100	100	129		100	100	89	
Ng.cntr. w/med.	100	100	109	1.0	100	100	89	1.0
pos.cntr.	13	58.1	505	3.9	89.8	51.6	367	4.1
5.0	116.2	92.8	161	1.5	91.1	97.3	98	1.1
10.0	105	81.4	175	1.6	103.3	118.5	95	1.1
20.0	108.5	79.6	188	1.7	88.4	98.4	93	1.0
100.0	88.4	56.5	194	1.8	95.4	105.4	103	1.2
320.0	55.8	71.6	154	1.4	67.1	92.5	81	.9
W/S9								
Ng.cntr.	100	100	101		100	100	100	
Ng.cntr. w/med.	100	100	113	1.0	100	100	100	1.0
pos.cntr.	88.3	64.1	274	3.9	68	65	328	3.3
5.0	103.3	112.1	127	1.5	101.7	83.8	169	1.7
10.0	101.6	99.4	96	1.6	109.3	112.1	104	1.0
20.0	101.6	103.9	116	1.7	105.4	131.5	126	1.2
100.0	80.8	136.8	118	1.8	77.6	94.6	99	1.0
320.0	73.0	83.1	129	1.4	26.2	20.2	202	2.0
Experiment 2								
Ng.cntr.	100	100	127		100	100	123	
Ng.cntr. w/med.	100	100	98	1.0	100	100	112	1.0
pos.cntr.	41.6	41.6	551	5.6	66.6	26.5	704	6.3
5.0	101.8	113.3	61	.6	92.4	125.9	73	.7
10.0	118.8	105.9	103	1.0	89.2	108.3	79	.7
20.0	100.0	114.6	64	.6	97.1	87.7	109	1.0
100.0	101.8	75.8	114	1.2	69.7	121.6	55	.5
320.0	87.4	99.5	90	.9	68.5	91.0	90	.8

Conclusions

Under the experimental conditions reported the test item did not induce mutations in the mouse lymphoma thymidine kinase locus assay using the cell line L5178Y in the absence and presence of metabolic activation.

Data Quality

Reliability:

Klimisch Code 1 Reliable without restriction

References

Daphnia

Test Substance

Test substance:	C.I. Pigment Red 122, CAS No. 980-26-7

Remarks:

Method

Method:	OECD 211
Test type:	Daphnia Magna Reproduction Test
GLP:	Yes
Year:	1999
Species/strain:	Daphnia Magna
Analytical monitoring:	Temperature: 20.8 to 21.2 degrees centigrade, total hardness 2.1 to 2.3 pH 8.0 to 8.2
Exposure period:	21 days
Remarks:	Semistatic system, the daphnids were transferred into fresh prepared test solutions on Monday, Wed. and Fri. Inspection and feeding of the daphnids tool place every 24 hours and involved recording the immobility, the reproduction rate and the development of embryos in the brood pouch. Immobile daphnids were removed from the chambers. Those animals not able to swim within 15 seconds after gentle agitation of the test container were considered to be immobile. The pH value oxygen content conductivity and total hardness were measured and recorded once a week at the beginning and at the end of a test interval in one representative test vessel of each concentration group. 1 daphnid per vessel 100 ml each. Negative control also tested.

Results

Nominal concentration:	1 mg/L
Measured concentration:	since solubility is below analytical detection concentration assumed to be < 20 ug/L
Endpoint value:	NOEC immobility 1mg/L solubility <20 ug/L NOEC reproduction 1mg/L
Biological observations:	solubility <20 ug/L
	no immobility in the 1mg/L nominal concentration group

Conclusions not toxic under the conditions of the study

Data Quality

Reliability:	Reliable without restriction, Code 1
Remarks:	

References

Other

D. Partition Coefficient

Test Substance

Test substance:	C.I. Pigment Violet 19, CAS No. 1047-16-1
Remarks:	

Method

Method:	Octanol Solubility Determination
Remarks:	2007 ETAD Method 229

Results

Solubility:	10.3 ug/L Water 1380 ug/L Octanol Log Kow 2.12
Remarks:	Log Kow Calculated from actual solubility analysis in water and octanol

References ETAD Results of solubility analysis

Reliable without Restriction Code 1

Other

E. Water Solubility

Test Substance

Test substance:	C.I. Pigment Violet 19, CAS No. 1047-16-1
Remarks:	

Method

Method:	Octanol Solubility Determination
Remarks:	2007 ETAD Method 229

Results

Value:	10.3 ug/L Water
Temperature:	Room Temperature
Description:	Pigment is stirred in 100ml water for approx. 3 days
Remarks:	Concentration in solution is measured directly in UV/VIS and spiked. The result is calculated over a regression curve.

References

ETAD results of representative solubility analysis

Other

Genetic Toxicity

Test Substance

Test substance: C.I. Pigment Violet 19 CAS No. 1047-16-1

Remarks: Purified Pigment

Method

Method: OECD 474
Test type: In Vivo - Rat hepatocyte unscheduled DNA synthesis Assay
GLP: Yes
Year: 1988
Species/strain: Fischer 344 Male Rats
Exposure period: 14 days
Remarks:

Procedure :

Group	Dose	No. animals	Killing time
1	0	4M	14 days
2	V19 1%	4M	14 days.
3	V19 5%	4M	14 days.
4	V19 10.0%	4M	14 days.
5	2AAF .02%	4M	14 days.
6	corn oil 0	3M	14 days.
7	2AAF 50	3M	14 days.

The appropriate test article diets or control diets were made available to the test animals for 14 consecutive days at which time the primary hepatocyte cultures were obtained from the animals. samples of selected test articles diets were analyzed as part of a related Subchronic Oral Toxicity study completed in the same laboratory.

The procedure used for obtaining rat hepatocyte cultures was essentially that of Williams et al. (In Vitro 13:809-817, 1977). Each rat used was sacrificed by inhalation of metofane. The animals were dissected and the liver perfused first with .5mM EGTA solution and then with collagenase solution. The liver was removed from each animal and the cells were dissociated, counted and seeded into 35 mm dishes ($5X10^5$ viable cells/ dish). The cells were seeded in Williams Medium E supplemented with 10% fetal bovine serum, 2mM L-glutamine, 100 units of penicillin and 100ug of streptomycin/ml or 50 ug/ml gentamicin (complete WME). Twelve cultures per rat, six containing coverslips (for the UDS assay) and six without (for parallel viability) were incubated at 37 +/- 1 ◦C in a humidified 5 +/- 1% CO_2 incubator.

Plates were washed after two hours with WME. the parallel viability cultures werer refed with serum free complete WME and the UDS cultures were refed with serum-free complete WME containing 10uCi/ml 3H-thymidine. Approximately four hours later, the UDS cultures were washed three times and refed with serum -free complete WME containing .25mM thymidine.

Seventeen to twenty hours after the 3Hthymidine containing medium was removed from the UDS cultrues, theree parallel viability plates were harvested. An aliquot of culture fluid was removed, centrifuged, and the level of lactate dehydrogenase (LDH) activity in the culture fluid determined. (The relative viabilities and relative toxicities were obtained by comparing the LDH activity in the treated and control cultures with the LDH activity in the 1000 Lysed cultures.)

Seventeen to twenty hours after the 3thymidine containing medium was removed, the cells in the Unscheduled DNA synthesis assay plates were washed in serum-free WME, swlled in 1% sodium citrate and fixed in ethanol-acetic acid dixative. The coverslips were air dried , mounted (cell side up) on glass slides , and allowed to dry. The slides were coated with Kodak NTB emulsion and stored for none days at 4 ∘C in light tight boxes with desiccant. The slides were then developed in Kodak D -19 developer, dixed in Kodak fixer and stained with hematoxylin-sodium acetate-eosin.

The slides were read "blind " on an Artek colony counter. Nuclear grains were counted in 50 cells in random areas of each of two cover slips per animal. The net nuclear counts were determined by counting three nucleus sized areas adjacent to each nucleus and subtracting the average cytoplasmic count from the nuclear count. Replicative synthesis was identified by nuclei completely blackened with grains and such cells were not counted. Nucei exhibiting toxic effects of treatment, such as dark or uneven staining, disrupted membranes or irregular shape were not counted.

For each slide the net nuclear counts were averaged and the standard deviation determined and recorded on a summary form. Also reported are the grand mean and std. deviation for each dose level as well as the pecent of cells in repair (cells with $\geq$ 5 net nuclear grains).

The results of the study were evaluated according to the following criteria. If the mean net nuclear count was increased by at least five counts over the control, the results for a particular dose level were considered significant. A test article was judged positive if it induced a dose related response and at least one dose produced a significant increase in the average net nuclear grain s when compared to that of the control. In the absence of a dose response, a test article which showed a significant increase in the mean net nuclear grain count in at least two successive doses was considered positive. If a test article showed a significant increase in the net nuclear grain count at one dose level without any dose response, the test article was considered to have a marginal positive activity. The test article was considered neg. if no significant increase in the net nuclear grain counts at any dose was observed.

Results:

An.#	Dose	Slide No.	Nucei counted	Av.net grains	S.D.*	Grand mean	SD*	%Cells≥ 5 grains
Quinacridone Violet 19								
213	10.0%	27a	50	-2.11	1.6	-1.9	1.5	0
		27b	50	-1.7	1.4			
214	10.0%	21a	50	-.8	1.3	-1.2	1.6	0
		21b	50	-1.6	1.9			
209	5.0%	22a	50	-1.7	1.6	-1.9	1.5	0
		22b	50	-2.2	1.3			
212	5.0%	30a	50	-2.2	1.6	-2.2	1.5	0
		30b	50	-2.2	1.4			
207	1.0%	25a	50	-2.2	1.6	-1.9	1.4	0
		25b	50	-1.5	1.3			
208	1.0%	26a	50	-.8	1.9	-1.4	2.0	0
2-acetylaminofluorine (2AAF)								
217	.02%	29a	50	7.4	2.6	7.2	2.4	81
		29b	50	6.9	2.2			
218	.02%	28a	50	6.8	3.0	8.1	3.0	83
		28b	50	9.5	2.3			
Purina Certified Rodent Chow								
201		24a	50	-1.6	2.0	-1.8	2.2	0
		24b	50	-1.9	2.4			
202		20a	50	-1.5	1.1	-1.8	1.5	0
		20b	50	-2.1	1.9			

Conclusions

The results of the UDS assay indicate that under the test conditions, one of the test article doses caused a significant increase in Unscheduled DNA synthesis in hepatocytes isolated from the treated animals. A significant increase is defined as an increase of a least 5 net nuclear counts over the control. In this study, the positive control, 2 acetylaminofluorene (2AAF) was also administered via nuclear grain counts over that in the negative control

Data Quality
Reliability:

Klimisch Code-1- Reliable without restriction

References

Acute toxicity

Test substance: C.I. Pigment Red 202 , 2,9 Dichloroquinaqcridone, CAS No. 3089-17-6

Remarks: Commercial Pigment Product 100%

Method

Method: Acute lethality; Single Dose Oral Toxicity in Rats
Test type: LD_{50} estimate
GLP: yes
Year: 1992
Species/strain: Wistar Albino Rat
Route of exposure: Oral gavage
Dose levels: 5 g/kg body weight
Remarks: 5 male and 5 female rats weighing 200-300 grams

The test article was added to corn oil inorder to make dosing by gavage possible. The dose was based on the dry wight of the test article. The test article dilution was administered orally, one time, by syringe and dosing needle at a dose level of 5 g/kg. The maximum volume of liquid administered at one time did not exceed 1.0 ml/100 g body wight for non aqueous vehicles and 2.0 ml/100 g body weight for aqueous solutions. Animals were observed 1,2 and 4 hours post dose and once daily for 14 days for mortality. Body weights were recorded immediately pretest, weekly and at termination.

Results

Animal	Sex	BW I	BW F.	Dose VOL. CC*
1	M	278	387	2.8
2	M	271	360	2.7
3	M	272	357	2.7
4	M	257	361	2.6
5	M	259	342	2.6
6	F	211	252	2.1
7	F	205	270	2.1
8	F	222	266	2.2
9	F	248	291	2.5
10	F	231	295	2.3

20 grams of test article were added to corn oil for a total volume of 40 ml.

Conclusions

All animals appeared normal throughout the 14 day test period. Nine autopsies were normal. A herniated liver protruding through the diaphragm was observed in animal no. 3. Rat no. 2 experienced diarrhea at hour 4, anogenital arear stained purple for all animals day 1 and day 2.
LD_{50} = >5,000 mg/kg,

Data Quality

Reliability: Reliable, well documented GLP Study. Klimish Code : 1

References

Acute toxicity

Test substance: C.I. Pigment Red 122 , 2, 9 Dimethylquinacridone, CAS No. 980-26-7

Remarks: Commercial Pigment Product 100% pure

Method

Method:

Test type: Acute lethality; Single Dose Oral Toxicity in Rats

GLP: LD_{50} estimate

Year: yes

Species/strain: 1992

Route of exposure: Wistar Albino Rat

Dose levels: Oral gavage

Remarks: 5 g/kg body weight
5 male and 5 female rats weighing 200-300 grams

The test article was added to corn oil inorder to make dosing by gavage possible. The dose was based on the dry wight of the test article. The test article dilution was administered orally, one time, by syringe and dosing needle at a dose level of 5 g/kg. The maximum volume of liquid administered at one time did not exceed 1.0 ml/100 g body wight for non aqueous vehicles and 2.0 ml/100 g body weight for aqueous solutions. Animals were observed 1,2 and 4 hours post dose and once daily for 14 days for mortality. Body weights were recorded immediately pretest, weekly and at termination.

Results

Animal	Sex	BW I	BW F.	Dose VOL. CC*
1	M	268	352	2.7
2	M	260	370	2.6
3	M	269	372	2.7
4	M	271	360	2.7
5	M	235	358	2.4
6	F	211	242	2.1
7	F	221	261	2.2
8	F	208	258	2.1
9	F	223	255	2.2
10	F	232	276	2.3

*20 grams of test article were added to corn oil for a total volume of 40 ml.

Conclusions

All animals appeared normal throughout the 14 day test period. All autopsies were normal. Rats no. 1,4, and 5 experienced diarrhea at hours 2 and 4, anogenital area stained purple for the same animals day 1 and day 2. LD_{50} = >5,000 mg/kg,

Data Quality

Reliability:

Reliable, well documented GLP Study. Klimish Code : 1

References

Redacted

Repeated Dose Toxicity

Test Substance

Test substance:	C.I. Pigment Violet 19, CAS No. 1047-16-1
Remarks:	97.3% pure

Method

Method:	Repeated subchronic dose
Test type:	
GLP:	NA
Year:	1982
Species/strain:	Fisher 344 Rats
Route of exposure:	Gavage
Duration of test:	33 days
Exposure levels:	Rats 0. 1.0%, 5.0 %,10.0% in the diet

Sex:

Exposure period:	33 days
Post-exposure Observation period:	none
Remarks:	Groups of 8 male and 8 female animals were dosed at 0%, 1%, 5% and 10 % with feed for 33 consecutive days. An extensive necropsy was performed under the supervision of the pathologist at day 34. The necropsy procedure was a thorough and systematic examination and dissection of the animal viscera and carcass. Brain, liver spleen, kidneys and testes or ovaries were weighed at scheduled necropsy. Adrenals and thyroids/ parathyroids were placed in a cassette at necropsy and weighed after at least 48 hours of fixation. Extraneous tissues were carefully removed from all organs prior to weighing. Paired organs were weighed together unless a gross lesion was present in one. Statistical Analysis: Analysis of variance tests were conducted on body weight, food consumption, hemotology, clinical chemistry and organ weight data. If a significant F ratio was obtained ($p \leq 0.05$), Dunnett's t test was used for pair-wise comparisons. Individual animal data is available. Frequency data such as incidence of mortality and gross necropsy observation were compared by Fisher's Exact Test or Chi-square analysis as necessary.

Results

NOAEL (NOEL):	up to 10 % in the diet

Up to 10 % of the diet

After repeated oral administration for 33 days in rats, pigment Violet 19 showed no signs of toxicity. None of the study animals died on test. Clinically, high dose (10%) animals demonstrated significant body weight gain compared to controls, which appeared to be associated with corresponding increase in food intake. It appeared that these animals tried to compensate by overeating for the decrease in nutritional intake in the 10% pigment diet. These animals, and to a lesser extent the 5% and 1% dose level animals, also had purple tinged fur, apparently as a result from coming in contact with the color pigment in feed hoppers. No other clinical sign were seen in the animals. Clinical pathology, ophthalmology, cytogenetic analysis, organ weights, and gross and tissue morphology examinations failed to detect the toxicity associated with Pigment Violet 19. (A very slight but statistically significant increase in methemoglobin levels was seen for the high dose female rats at week 2, but in neither sex at week 4. Not considered related to Pigment Violet 19 treatment.) In general, under the conditions of the study, toxicity was not observed following the administration of up to 10% Pigment Violet 19 in the diet of Fisher 344 rats for 33 days.

The following tissues and organs were examined histologically for all high dose and control animals :

Mandibular and mesenteric lymph node	**Ileum**
Sternum including marrow	**Colon**
Lungs and bronchi	**Cecum**
Heart and Aorta	**Liver**
Thyroid	**Kidneys**
Parathyroids	**Adrenals**
Stomach	**Urinary Bladder**
Duodenum	**Testes, including epididymis**
Jejunum	**Ovaries**
Salivary Gland	**Brain**
All gross lesions	**Mammary Gland**

Conclusions Test substance is not toxic

Data Quality

Reliability: Reliable without restriction, Code 1

Remarks:

References:

Other

Repeated Dose Toxicity

Test Substance

Test substance: Quino(2,3-b)acridine-7,14-dione,5,12-dihydro

Remarks:

Method

Method: A Radiolabeled Tracer Study of ^{14}C-Quinacridone-Violet 19 in the Albino Rat

Test type:

GLP: NA

Year: 1991

Species/strain: Fisher 344 Rats

Route of exposure: Gavage

Duration of test: 72 Hours

Exposure levels: 3.22 mg/kg and 33.68 uCi/kg Males, 5.44mg/kg 56.81 uCi/kg Females

Sex:

Exposure period: single dose

Post-exposure Observation period: 72 hour follow up

Remarks:

Results

NOAEL (NOEL): N/A

The test article was administered to 4 male and 5 female rats as a suspension in aqueous 1% carboxymethyl cellulose at a concentration of .3905 mg QV19 and the same amount was administered to each rat. Urine and feces were collected from each rat at 2,8,24,48 and 72 hours after dosing; cage washes and gastrointestinal tract of each rat were removed after euthanasia at 72 hour post-dose. Recovery of administered radioactive dose was virtually complete.91.9+ or - 6.9 % of dose males; 100.5+ or_8.7% of dose females. There were no gender related differences in the route of excretion. More than 90 % of the recovered radioactivity was eliminated in the feces and cage washes, which appeared to contain residual fecal matter. At 72 hours virtually all radioactivity had been eliminated by the rats. The urine from both groups of rats contained very low amounts of radioactivity.0089% of dose males;.0020% of dose females.

Conclusions Radioactivity from a single oral dose of Pigment Violet 19 given to male and female rats was eliminated almost completely in the feces.

Data Quality

Reliability: Code 1, Reliable without restriction

Remarks:

References:

Repeated Dose Toxicity

Test Substance

Test substance: Quino(2,3-b)acridine-7,14-dione,5,12-dihydro

Remarks:

Method

Method: Whole Body Radiography

Test type:

NA

GLP: 1991

Year: Fisher 344 Rats

Species/strain: Gavage

Route of exposure: 48 Hours

Duration of test:

Exposure levels:

Sex: single dose

Exposure period: 48 hour follow up

Post-exposure Observation period:

Remarks:

Results N/A

NOAEL (NOEL): Groups of 4 male and 4 female Fisher 344 rats were administered orally by gavage pigment violet 19 and radioactive trace material. And the tissue distribution of radioactivity determined by whole body autoradiography at selected times up to 48 hours after dosing. The autoradiogram showed that radioactivity was localized only in the gastrointestinal tract of both male and female rats. No radioactivity was detected in other organs and tissues of the animals. The highest concentrations of radioactivity were found at 2 hours post dosing . Most of the radioactivity was eliminated from the rats at 2-4 hours and it was virtually undetected at 18 hours post-dose.

Conclusions Whole body autoradiography indicated that virtually no radioactivity was detected in tissues, supporting the previous finding that, radioactivity from a single oral dose of Pigment Violet 19 given to male and female rats was eliminated almost completely in the feces.

Data Quality

Reliability: Code 1, Reliable without restriction

Remarks:

References:

Other